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COMPARISONS OF *IN VITRO* PROPAGATION OF TOMATO CULTIVARS

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ABSTRACT

Comparisons were made between *in vitro* propagation of Cal-j, Petomech and Red Cloud cultivars of tomato (*Licopersicon esculentum* Mill.). Shoot tips, excised from greenhouse-grown plants, proliferated subterminal buds on a modified Murashige and Skoog (1962) salts medium (MS) plus (in mg 1^{-1}): nicotinic acid, 0.5; pyridoxin HCl, 0.5; thiamine HCl, 0.1; glycine, 0.2; inositol, 100; sucrose, 30,000, and Bacto agar 4,000, supplemented with cytokinins and without auxins.

In presence of benzyladenine (BA) or kinetin (K), Cal-j had more shoot proliferation compared to the other two cultivars. The best cytokinin concentration for Cal-j was either 1.5 mg 1^{-1} BA or 3 mg 1^{-1} K. At these concentrations, on average, 9.62 and 8.28 shoots per explant were obtained, respectively. For Petomech and Red Cloud, optimum cytokinin concentrations were either 2 mg 1^{-1} BA or 5 mg 1^{-1} K.

Well-branched roots were obtained when micro cuttings were transferred to MS medium containing only auxins as growth regulators. Indolebutyric acid (IBA) was superior to naphthaleneacetic acid (NAA); 0.5 mg 1^{-1} IBA being optimum. The concentration of 4 g 1^{-1} agar was found suitable for rooting.

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مقایسه ازدیاد درون شیشه‌ای ارقام گوجه‌فرنگی

معصومه ایزدپناه و مرتضی خوشخوی
به‌ترتیب دانشجوی سابق کارشناسی ارشد و استادبخش باغبانی دانشکده کشاورزی دانشگاه شیراز

چکیده

مقایسه‌ای بین ازدیاد درون شیشه‌ای رقم‌های کلجی، پتومک و رد کلود گوجه‌فرنگی صورت گرفت. نوک شاخساره‌های جدا شده از گیاهانی که در گلخانه پرورش یافته بودند بر روی محیط کشت موراشیگی و اسکوگ (۱۹۶۲) حاوی نمکهای این محیط همراه با (به میلی گرم در لیتر): اسیدنیکوتینیک ۰/۵، پیرودوکسین HCl ۰/۵، تیامین HCl ۰/۱، گلیسین ۰/۲، اینوزیتول ۱۰۰، ساکاروز ۳۰۰۰۰، و باکتوآگار ۴۰۰۰، که بدان سایتوکاینین (با یا بدون اکسین) افزوده شده بود، جوانه‌های زیرانتهایی، پرآوری کردند.
در حضور بنزیل آدنین یا کینیتن، رقم کلجی پرآوری شاخساره بیشتری در مقایسه با دو رقم دیگر داشت. بهترین غلظت سایتوکاینین برای کلجی ۱/۵ میلی گرم در لیتر بنزیل آدنین یا ۵ میلی گرم در لیتر کینتین بود.
هنگامی که ریزقلمه‌ها بر روی محیط کشت موراشیگی و اسکوگ حاوی اکسین به تنهایی بعنوان گنارای گیاهی قرار گرفت، ریشه‌های کاملا منشعبی بدست آمدند. اسیدایندول بوتیریک بهتر از اسیدنفتالین استیک و ۰/۵ میلی گرم در لیتر اسیدایندول بوتیریک، بهینه بود. غلظت مناسب آگار برای ریشه‌زایی ۴ گرم در لیتر بود.

INTRODUCTION

In vitro propagation of tomato (*Lycopersicon esculentum* Mill.) has been the subject of a number of studies (1, 3, 5, 8, 9).

Padmanabhan, *et al.* (9) reported shoot regeneration in tomato explants from leaf-derived callus cultures. To accelerate vegetative propagation of this crop, De Langhe and De Bruijne (3) used callus obtained *in vitro* from stem internode tissues. Kartha, *et al.* (4) described the growth and morphological response of tomato explants to various combinations of auxins and cytokinins. Tomato propagation through *in vitro* apical meristem and shoot-tip culture has been investigated (5, 7), and the effects of cytokinins and auxins on growth and development of these explants have been tested.

In the present study, comparative growth regulator requirements for shoot multiplication and rooting of three tomato genotypes were determined.

MATERIALS AND METHODS

Three tomato cultivars, Cal-j, Petomech and Red Cloud, which are widely under cultivation in Iran, were used in this investigation. Shoot-tips were taken from eight-week-old greenhouse-grown plants. They were immediately transferred to the laboratory and held in tap water. The explants were surface sterilized by immersion in 10% Clorox (5.25% NaOCl) for 10 min, then rinsed three times in autoclaved water. The lateral leaves were removed and the terminal 5-10 mm of the shoot-tip excised. Explants were cultured in 56 ml round glass bottles (one in each) containing 25 ml of Murashige and Skoog (6) (MS) medium salt concentrations plus (in mg 1^{-1}) nicotinic acid, 0.5; pyrodoxine HCl, 0.5; thiamine HCl, 0.1; glycine, 0.2; inositol, 100; sucrose, 30,000; Bacto agar 4,000, supplemented with different concentrations of growth regulators. The pH of all media was adjusted to 5.8 with 0.1 N HCl before autoclaving. Media were sterilized by autoclaving for 20 min at 120°C at 1.5 kg cm^{-2} pressure.

The glass bottles containing explants were placed under a 16-h photoperiod of 2 klux light intensity provided by cool white fluorescent lamps at 26 ± 2°C temperature. On the basis of a series of preliminary experiments, proliferation media containing no auxins but with concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg 1^{-1} benzyladenine (BA) or 1, 2, 3, 4 and 5 mg 1^{-1} kinetin (K) were tested.

Root formation was studied by transferring excised shoot-tips from multiplication cultures to glass vials containing MS salts and vitamins, 3% sucrose and 2, 4, 6 and 8 g 1^{-1} agar with no cytokinins and 0, 0.2, 0.5, 1.0 and 2.0 mg 1^{-1} NAA or IBA.

Data for multiplication rate and rooting percentage were taken after four and three weeks,

respectively. For shoot proliferation, a four-week subculture interval was maintained.

To acclimatize *in vitro* rooted plants to planting medium, they were gently removed from the culture medium, washed free of agar and transplanted into a pasteurized 1/3 loam soil, 1/3 sand and 1/3 peat moss (v/v/v) medium. Initially, they were irrigated frequently with distilled water and kept under a plastic tent for about two weeks. Subsequently, they were transplanted to the same soil mixture in pots and kept in a greenhouse at 16± 3°C. High humidity was maintained by moist sand under the pots and frequent misting during the first days after transfer. After two weeks in the greenhouse the success of acclimatization was recorded.

All experiments were carried out as a completely randomized design with at least eight replications. Comparisons of means were made by using Duncan's multiple range test.

RESULTS

Shoot Proliferation

Shoot arose directly on explants or from callus that formed at the base of that shoot tip explants. For both cytokinins, the average number of shoots per explant produced by three cultivars was significantly different. Table 1 shows the data averaged over the different concentrations of BA and K. Overall, Cal-j produced significantly more shoots per plant as compared with the other two cultivars.

Growth Regulators

The best cytokinin concentration for Cal-j was either 1.5 mg l^{-1} BA which resulted in 9.62 shoots per explant, or 3 mg l^{-1} K which produced 8.28 shoots per explant (Fig. 1). Petomech and Red

Table 1. Average number of shoots produced per explant in three tomato cultivars across all K and AB levels.

Cytokinin	Cultivar		
	Petomech	Red Cloud	Cal-j
K	2.84aA†	3.54aB	5.61aC
BA	4.72bB	4.32bA	6.09aC

†Means with common letters (capital letters in rows and small letters in columns) are not significantly different at 1% level of probability using DMRT.

Cloud produced the highest number of shoots per explant, 7.16 and 5.34, respectively, at 2 mg 1^{-1} BA. The optimum K concentration for these cultivars was 5 mg 1^{-1} with 4.94 and 4.91 shoots per explant for Petomech and Red Cloud, respectively. In general, BA was superior to K for the cultivars under the study.

Subcultures

Comparisons of original culture with three subsequent subcultures of each cultivar showed that in the presence of K, Cal-j produced higher number of shoots per plant in the first and second subcultures as compared with the original culture (Table 2). The same observations were made for Petomech when BA was used. But, generally, there was a gradual decrease in number of shoots per plant in further subcultures of each cultivar.

Rooting

There was a difference between cultivars as far as rooting was concerned. Red Cloud rooted better

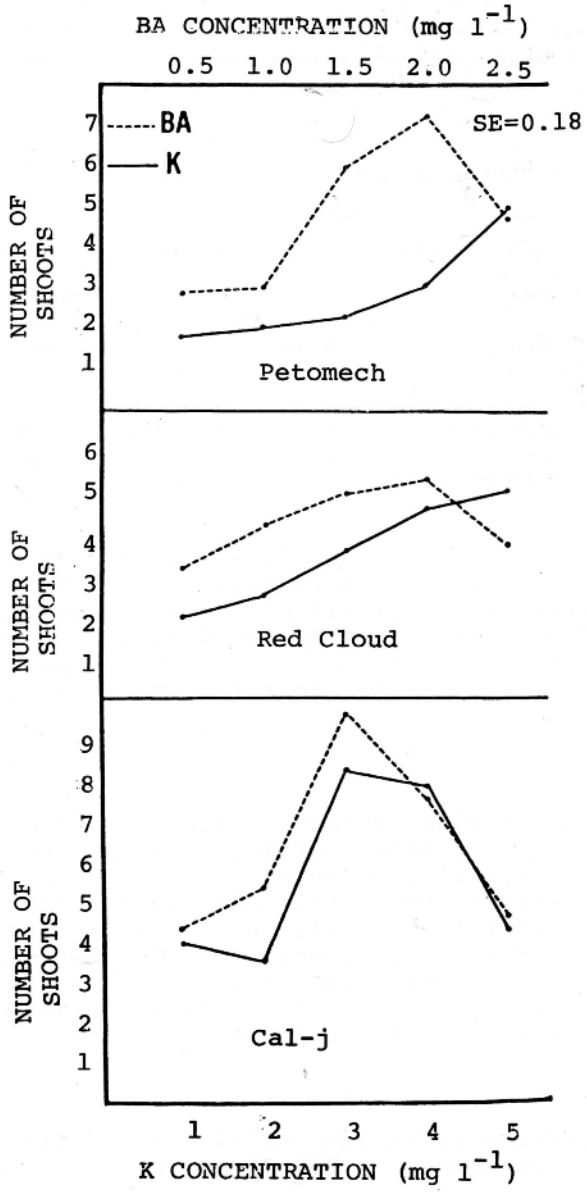

Fig. 1. Effects of different concentrations of BA and K on number of shoots per plant in three cultivars of tomato.

Table 2. Average number of shoots produced in original culture and through three subsequent subcultures for three tomato cultivars.

Cultures	K			BA		
	Petomech	Red Cloud	Cal-j	Petomech	Red Cloud	Cal-j
Original culture	2.85a	4.32a	2.99a	4.03a	5.07b	7.35c
First subculture	2.93a	3.50a	7.85c	5.50a	5.03b	7.50c
Second subculture	3.33a	3.83a	5.80b	5.70a	3.50a	5.55b
Third subculture	2.25a	2.70a	5.08ab	3.85b	3.67a	3.88a

Means with common letter are not significant at 1% level, using DMRT.

than the other two cultivars. Among the agar concentrations used, 4 mg l^{-1} was the best concentration. Explants on multiplication media produced roots so that some propagules particularly those taken from MS medium containing low concentrations of K, showed slight rooting. Explants left on proliferation media for more than two months produced a complete root system (Fig. 2). However, roots produced on MS medium containing cytokinins were, in general slender and elongated thus not suitable for transfer to the planting medium. Propagules placed on media containing auxins but no cytokinins produced well-branched root systems, which further transferred successfully to soil. In general, IBA was superior to NAA for tomato rooting. The highest percentage of rooting obtained was with 0.5 mg l^{-1} IBA (Fig. 3).

DISCUSSION

The results obtained in this study showed that tomato cultivars responded differently on *in vitro* propagation. Novak and Maskova (7) using cv. Money Maker attributed some differences in results obtained with those of Kartha, *et al.* (4) who used cv. Starfire to cultivar differences. Behki and Lesley (1) used leaf discs as explants and tested 15 cultivars and among them only 12 cultivars were regenerated. Ohki, *et al.* (8) used hypocotyl segments as explants and reported that cv. Prophyr was always superior to cv. Apedice. We used shoot-tips in this experiment and obtained significant differences between cultivars for their growth regulator requirements in micropropagation.

Our results indicated that tomato shoots proliferated only in the presence of cytokinins. Endogenous auxin was probably responsible for this reaction as Cassells (2) used TIBA on tomato plants and obtained higher shoots on stem segments. Also, inhibiting effect of auxins on *in vitro* propagation of tomato has been reported by others (7, 10).

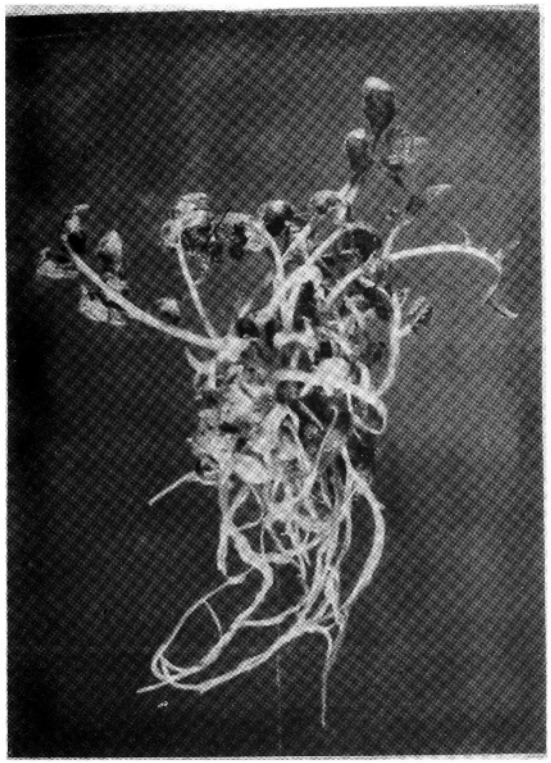

Fig. 2. Root formation of Cal-j on multiplication medium.

Fig. 3. Well-branched roots of Cal-j on MS medium supplemented with 4 g l^{-1} agar and 0.5 mg l^{-1} IBA.

According to data obtained in the present investigation, to ensure satisfactory root formation, it was necessary to transfer propagules to an MS medium containing auxins and free from cytokinins. This is in accordance with results reported by Novak and Maskova (7).

ACKNOWLEDGEMENT

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LITERATURE CITED

1. Behki, R.M. and S.M. Lesley. 1976. *In vitro* plant propagation from leaf explants of *Lycopersicon esculentum* (tomato). Can. J. Bot. 54:2409-2414.

2. Cassells, A.C. 1979. The effect of 2, 3, 5-triiodobenzoic acid on caulogenesis in callus cultures of tomato and Pelargonium. Physiol. Plant. 46:159-164.

3. De Langhe, E. and E. De Bruijne. 1976. Continuous propagation of tomato plants by means of callus cultures. Scientia Hortic. 4:221-227.

4. Kartha, K.K., O.L. Gamborg, J.P. Shyluk and F. Constable. 1976. Morphogenetic investigations on *in vitro* leaf culture of tomato (*Lycopersicon esculentum* Mill. cv. Starfire) and high frequency plant regeneration. Z. Pflanzenphysiol. 77:292-301.

5. Kartha, K.K., S. Champoux, O.L. Gamborg and K. Pahl. 1977. *In vitro* propagation of tomato by shoot apical meristem culture. J. Amer. Soc. Hortic. Sci. 102:346-349.

6 Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15:473-479.

7. Novak, F.J. and Irena Maskova. 1979. Apical shoot-tip culture of tomato. Scientia Hortic. 10:337-344.

8. Ohki, S., C. Bigot and J. Mousseau. 1978. Analysis of shoot-forming capacity *in vitro* in two lines of tomato (*Lycopersicon esculentum* Mill.) and their hybrids. Plant & Cell Physiol. 19:27-42.

9. Padmanabhan, V., E.F. Paddock and W.R. Sharp. 1974. Plantlet formation from *Lycopersicon esculentum* leaf callus. Can. J. Bot. 53:1428-1432.

10. Pence, V.C. and J.L. Caruso. 1982. Effects of IAA, IAA conjugates and sucrose on morphogenesis from normal and lanceolate tomato *in vitro*. Plant Physiol. 69(Suppl.):73.